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FURTHER STUDIES ON THE DEGENERATION OF MONOAMINE NERVES IN THE VENUS CLAM HEART INDUCED BY NEUROTOXIC DRUGS: EFFECTS OF 5,7-DIHYDROXYTRYPTAMINE (5,7-DHT).

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SUMMARY

The long term effects of 5,7-DHT on nerves in the Venus clam heart were studied to elucidate further the structure of serotonergic nerves detected earlier.

At least 2 types of monoaminergic nerves with dense-cored vesicles were susceptible to significant degeneration by the drug. Among these were profiles with large granular vesicles (64-200 nm) which resemble possible serotonergic nerves seen in myenteric plexus and 'catch' muscle of *Mytilus*. The other nerves lesioned were those with small granular vesicles (32-64 nm) conforming to adrenergic nerves in vertebrates. A third type of profile with a population of medium-sized, dense-cored vesicles (40-160 nm) was found to be reactive and less susceptible to degeneration.

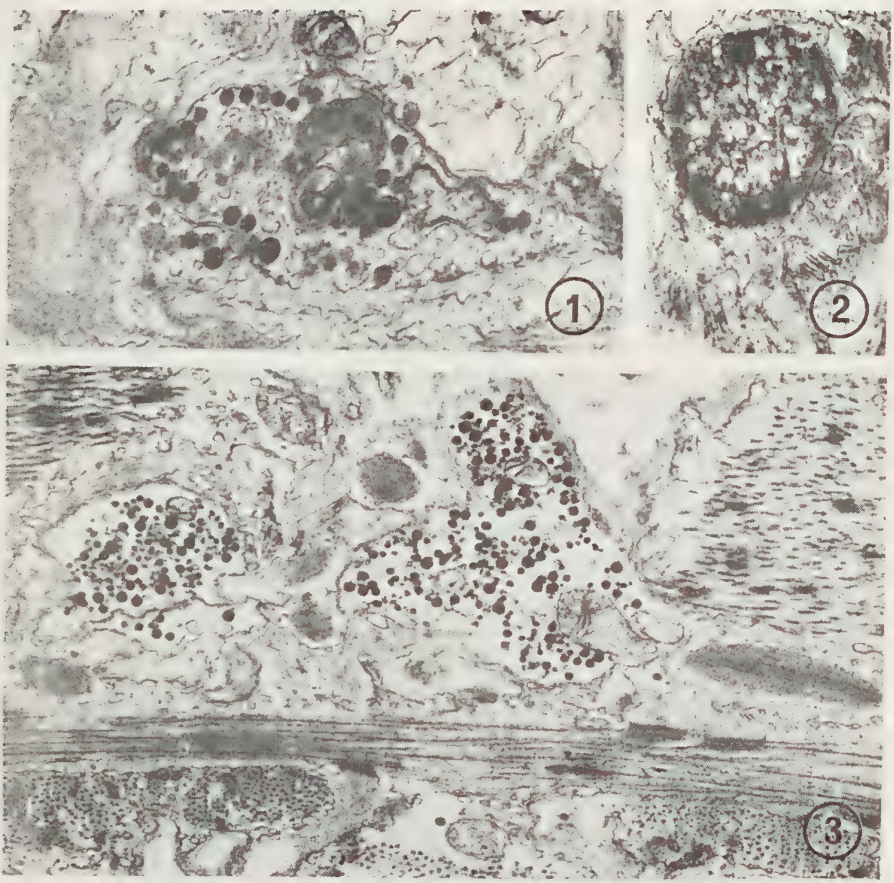
Nerves with a predominance of small agranular vesicles and large opaque vesicles were unaffected by the drug. These resemble vertebrate cholinergic and "purinergic" nerves, respectively.

The results confirm our previous studies on the molluscan heart with neurotoxic drugs.

Key words: Venus clam heart - *Katelysia rhytiphora* (Mollusca) - 5,7-Dihydroxytryptamine - 5-Hydroxytryptamine - Dopamine - Electron microscopy

INTRODUCTION

An earlier communication (Sathananthan & Burnstock, 1976) reported some degeneration of monoamine nerves in the heart of the Venus clam, induced by 5,6-Dihydroxytryptamine (5,6-DHT). Among these, were profiles with large granular vesicles (LGV) that were reactive to this drug and suspected to be serotonergic. 5,6-DHT also caused significant degeneration of similar nerves in *Mytilus* 'catch' muscle (Sathananthan, 1976).

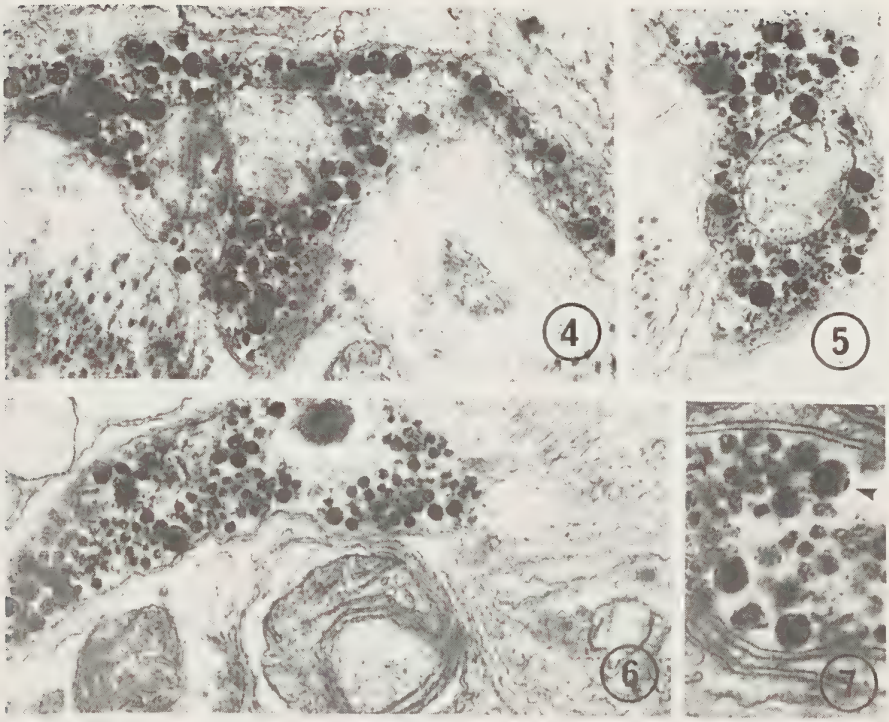


FIGURES 1-4. Electron micrographs of nerves in the clam heart after treatment with 5,7-DHT (8 days).

1. Degenerating non-terminal axon containing LGV (80-180 nm), some of which are enclosed in myelin-like membranes while others appear less electron-dense or degranulated. X28,000.
2. Non-terminal axon with ghosts of predominantly SGV (32-64 nm) and a few LGV (80-96 nm) in a denser axoplasm. X27,300.
3. Reactive profile containing MGV (40-140 nm) close to muscle. The larger vesicles have lost their haloes while the smaller vesicles have acquired cores indicating uptake of the drug. X15,400.

The dihydroxytryptamines are now being widely used in neurotoxic studies in mammals (see Baumgarten et al., 1974; Björklund et al., 1974), and 5,7-DHT is thought to be an improvement on 5,6-DHT in the lesioning of indoleamine nerves (Baumgarten & Lachenmayer, 1972). 5,7-DHT also produced extensive lesioning of possible serotonergic (5-HT) nerves in the myenteric plexus of *Mytilus* (Sathananthan, 1977a).

This paper deals with the long term effects of 5,7-DHT on nerves in the clam heart and a further attempt is made to establish the ultrastructural identity of 5-HT nerves in molluscs.



FIGURES 4-7

4. Terminal axon with reactive MGV (48-140 nm) wedged between muscle fibres forming close myoneural junctions. Note distorted mitochondria and denser axoplasm. X28,000.
5. Reactive terminal axon (vesicles 40-180 nm) between muscle fibres. X28,000.
6. Reactive axon (vesicles 40-160 nm) in a multiaxonal profile showing partial degeneration. An unreactive axon is seen on the right. X28,000.
7. Nerve with reactive vesicles showing denser crescents (arrow) and fine granules associated with their membranes. X51,000.

MATERIALS AND METHODS

Young venus clams, *Katelysia rhytiphora,* were collected and treated with 2.5 x 10^{-4} gm/ml 5,7-DHT as outlined in our previous study (Sathananthan & Burnstock, 1976). After 8 days of drug treatment, the hearts were fixed in 4% glutaraldehyde in 0.1M cacodylate buffer (pH 7.3) containing 8% sucrose (2 hr) followed by fixation in 1% OsO4 (1 hr) and embedded in Araldite. Sections ($\simeq$ 70 nm thick) were cut with a Reichert OMU3 ultramicrotome, stained with alcoholic uranyl acetate and alkaline lead citrate and examined with a Philips 301 electron microscope.

RESULTS

The appearance of various nerve types in controls and after short-term treatment with 5,6-DHT and 6-OHDA were reported earlier (Sathananthan & Burnstock, 1976). Axons were sometimes associated with Schwann-like cells and were often accompanied by glial processes. Multiaxonal profiles with different types of axons and nerves forming close myoneural junctions were commonly seen in this study.

DRUG EFFECTS

After 8 days treatment with 5,7-DHT well marked degenerative changes were noted in monoamine nerves, particularly in those with LGV, confirming our earlier observations with 5,6-DHT (Sathananthan & Burnstock, 1976). There were evidently two populations of LGV - one more susceptible to degeneration while the other was reactive and rather resistant to degeneration. Apart from these nerves, there were also profiles with small granular vesicles (SGV) that were also lesioned

The profiles with LGV that showed significant degeneration (Fig. 1) had vesicles (64-200 nm), distorted mitochondria, dense polymorphic and multivesicular bodies resembling lysosomes and phagosomes, respectively. Most of the LGV had lost their haloes and some appeared less electron-dense or degranulated. Clumps of LGV enclosed by myelin-like membranes were also seen.

The nerves that were most reactive to the drug were widely distributed and contained a range of medium-sized, granular vesicles (MGV), 40-160 nm, with many large vesicles (Figs. 3-7). Their axoplasms were often denser and the vesicles had lost their haloes or acquired specks or blobs of more electron-dense material and appeared to be labelled rather than loaded. Thus, their overall appearance seemed to be somewhat different to those nerves with LGV that were susceptible to degeneration by 5,7-DHT in this and previous studies where the material was processed in the same manner (Sathananthan, 1977a). Closer examination revealed that even smaller vesicles were granulated while some of the larger ones had 1 or 2 denser crescents associated with their membranes (Fig. 7). A few vesicles, both large and small, also had eccentric cores. The reactive axons were rather resistant to degeneration although a few showed signs of partial degeneration with clumps of vesicles (Fig. 6) or dense bodies.

The degenerating nerves with SGV (32-64 nm) showed typical signs of dark degeneration characteristic of catecholamine nerves. These profiles were either uniformly dense and revealed faint outlines of vesicles or had ghosts of SGV and a few LGV in a dense axoplasm (Fig. 2). Many were embedded in muscle and often associated with groups of muscle mitochondria.

Two types of nerve profiles remained unaffected by the drug. Of these, one had a predominance of small agranular vesicles (SAV), 40-70 nm, and were rare, while the other had large opaque vesicles (LOV), 80-200 nm, and were quite common and often large.

Apart from the above nerve types, there were large axons with only mitochondria and irregular specks of granular material resembling glycogen or huge profiles with numerous mitochondria packed with a heterogeneous population of LGV. Purkinje-like fibres similar to those found in the mammalian heart were also seen in the auriculoventricular junction of this clam heart.

DISCUSSION

The evidence presented confirms our earlier suspicions that nerve profiles in the molluscan heart containing LGV could possibly be serotonergic (Sathananthan & Burnstock, 1976). In fact, there seem to be two populations of large dense-cored vesicles that react differently to 5,7-DHT - one showing a greater susceptibility to degeneration than the other. Whether these represent two types of monoaminergic nerves is debatable.

The profiles that showed substantial degeneration resemble possible serotonergic nerves containing LGV identified with the dihydroxytryptamines in *Mytilus* 'catch' muscle and gut (Sathananthan, 1976, 1977a). All these nerves had dense-cored vesicles (56-200 nm) with a predominance of LGV. Similar degenerating nerves were also observed after treatment with 5,7-DHT in the myenteric plexus of this Venus clam (Sathananthan, 1977b) and in the CNS of *Helix* (Sathananthan, in preparation), where many of the axons had numerous degranulated LGV (65-225 nm). Such nerves could be serotonergic as there is also histochemical, biochemical and physiological evidence to indicate that 5,7-DHT is neurotoxic for indoleamine nerves in the snail brain (Osborne & Pentreath, 1976). Further, axons with LGV ($>$ 200 nm) were labelled with radio-active 5-HT in the CNS of *Helix* (Pentreath, 1976).

The axons with MGV that were less susceptible to degeneration but reactive, appeared to be loaded, suggesting uptake of the drug. The maximum diameter of these vesicles was usually $\sim$160 nm although one or two vesicles measured up to $\sim$180 nm. Axons with a similar population of vesicles were lesioned by 6-OHDA (personal communication) and these could be catecholaminergic. Comparable profiles with medium-sized granular vesicles (56-130 nm) were found to show less degenerative changes with 5,7-DHT in the myenteric plexus of *Mytilus* (Sathananthan, 1977a) but these were different in their overall vesicular appearance. Axons with reactive MGV were also

seen in the brain of *Helix*, side by side with nerves containing degranulating MGV, after injection of 5,7-DHT (Sathananthan, in preparation). Pentreath (1976) labelled similar nerve terminals with MGV (~ 100 nm) in the CNS of *Helix*, by injecting radioactive 5-HT. Whether the reactive profiles represent catecholaminergic or serotonergic nerves needs to be clarified by further study.

The degenerating axons with SGV resemble adrenergic nerves commonly seen in vertebrates. Similar nerves were affected by both 6-OHDA and 5,6-DHT in this heart (Sathananthan & Burnstock, 1976) and were thought to be dopaminergic. The dihydroxytryptamines lesion both serotonergic and adrenergic nerves in vertebrates (see Baumgarten et al, 1974; Bjorklund et al, 1974). The pharmacological, biochemical and histochemical evidence available indicates that 5-HT and DA are the possible monoaminergic neurotransmitters in the nervous system of bivalves, whereas evidence for the involvement of noradrenalin is lacking (see Sathananthan & Burnstock, 1976, for a detailed discussion). Therefore it seems logical to conclude that the two types of nerve profiles with LGV and SGV could represent serotonergic and catecholaminergic nerves in these molluscs.

The profiles with SAV that were not lesioned by 5,7-DHT conform to cholinergic nerves of vertebrates. Acetylcholine is now well established as a molluscan neurotransmitter and similar profiles with SAV were seen in our previous studies (Sathananthan & Burnstock, 1976; Sathananthan, 1976, 1977a). A further type of nerve terminal seen in all our studies with molluscan neurones were those with a predominance of LOV which resemble "purinergic" nerves in the vertebrate gut (see Burnstock, 1975). These findings confirm the presence of a non-cholinergic, non-monoaminergic innervation in this mollusc (Sathananthan & Burnstock, 1976), where the heart was seen to be sensitive to ATP and nerves with LOV were found to be unaffected by both 6-OHDA nad 5,6-DHT and showed Mg-ATPase and 5'nucleotidase activities.

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